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Phytochemical analysis and antifungal activity of *Azadirachta indica* and *Balanites aegyptiaca* seed extracts against *Fusarium oxysporum* isolate on tomatoes

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Fusarium oxysporum is the causative agent of tomato wilt and is an important disease of tomato crops worldwide. Management through chemical fungicides causes serious environmental damage and human health. This study assesses the antifungal potential of biochemical compounds of *Azadirachta indica* and *Balanites aegyptiaca* seeds extracts identified by GC-MS analytical method against *F. oxysporum*. Acetone, methanol and aqueous extracts of *A. indica* and *B. aegyptiaca* at 15, 30 and 60 µL/mL concentrations and synthetic fungicide (3.33 g/L) were tested *in vitro* in triplicate. Mycelial growth and Minimum inhibitory concentrations (MIC_{50}, MIC_{90}) were evaluated 8 days after inoculation (DAI). As results, 55 and 34 phytochemical compounds were identified in seed extracts of *A. indica* and *B. aegyptiaca* respectively. Methanol extract of *A. indica* and *B. aegyptiaca* at 60 µL/mL concentration showed a total inhibition (100%) of *F. oxysporum* mycelial growth. Low MIC50 values were obtained with aqueous extract of *A. indica* (8.77 µL/mL) and methanol extract of *B. aegyptiaca (*17.91 µL/mL). The tentative action mode showed that the methanol extract of both plants induced wall lysis of *F. oxysporum.* These results suggest that *A. indica* and *B. aegyptiaca* seed extracts have potent antifungal activity and could be used as an alternative to control *F. oxysporum*.

Keywords: Neem, desert date, bioactive compounds, mycelial growth, antifungal activity, *Fusarium oxysporum.*

INTRODUCTION

Tomato (*Lycopersicon esculentum* L.) is the most important and economic crop for the low-income farmers in the tropics (Prior *et al.*, 1994; Rizwana *et al.*, 2021). In Africa, tomatoes are the main source of income due to their short growth cycle, and can be harvested more than four times a year (Lima *et al.*, 2004). However, they are susceptible to diseases that affect their crops. Diseases induced by phytopathogenic fungi are considered a great economic loss in terms of production yield losses up to 100% in tomato crops due to their persistence and facility dissemination in the soil (Chowdappa *et al.*, 2013). *Fusarium oxysporum* f. sp. *lycopersici*, the causative agent of fusarium wilt of tomato is one of the most important diseases of tomatoes worldwide (Cal *et al.*, 2004; Hanaa *et al.*, 2011). *Fusarium oxysporum* can be transmitted from any part of the plant, as well as through soil-borne, and air-borne (Rongai *et al.*, 2017; Summeral *et al.*, 2003). Plants infected with *F. oxysporum* show symptoms such as necrosis, chlorosis, premature leaf drop and death of the plant (Ramsamy *et al.*, 1996). *F. Oxysporum* causes vascular systems resulting in wilting by infecting the roots and growing internally through the cortex to the stele (Bowers and Locke, 2000).

Control of *Fusarium* wilt depends on resistant tomato cultivars, crop rotation and mainly the use of effective synthetic fungicides, but these measures have been proven unsuccessful (Agbenin *et al.*, 2004; Jaiswal *et al.*, 2015). Furthermore, synthetic pesticides are known to have toxic effects on human health and the environment and may cause the selection of pathogen resistance (Lumsden and Locke, 1989; El-Mohamedy *et al.*, 2013; Barari, 2016). Many study have been carried out to find alternative methods of disease control using naturally occurring compounds derived from plant sources including extracts (Tegegne *et al.*, 2007;

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Hassanein *et al.,* 2010), which are less toxic, economical and highly efficient (Alabouvette, 1999; Parvu *et al*., 2011).

Plant extracts have received increased attention in recent years for the development of safe and environmentally friendly integrated crop management (ICM). Natural bioactive compounds used as pesticides are non-specific and their effect on pathogens is comprehensive (Isman, 2006). Plant extracts are rich in secondary metabolites (phenolic compounds, terpenoids and nitrogen compounds) that have been reported to possess pesticide properties to control crop diseases and pests. They have already successfully demonstrated their effectiveness (Meena *et al.,* 2021; Rabach *et al.,* 2022; Djeugap *et al.,* 2023). In addition, various chemicals compounds are present in plant extracts, including antioxidants, plant hormones and osmoprotectants, which activate plant defense mechanisms enabling them to resist abiotic and biotic stresses and play a crucial role in promoting plant growth (Fallanaj *et al.,* 2015; Desoky *et al.,* 2019). It is speculated that the antifungal properties of phenolic compounds are attributed to their lipophilicity property and the presence of the hydroxyl groups in their structure. Phenolic compounds are capable of disrupting membranes and inactivating enzymes, thus producing toxic effects upon pathogenic fungi. Indeed, lipophilicity facilitates cytoplasmic membrane penetration, whereas hydroxyl groups are involved in uncoupling oxidative phosphorylation (Acheuk *et al.,* 2022). According to Prasannath (2017), plant extracts could induce systemic resistance by activating the body's defenses, thus halting the spread of disease. Neem (*Azadirachta indica* L.), a tree in the Meliaceae family is used for its medicinal properties (Hossain *et al.,* 2005). Guleria and Kumar (2006); Aboellil (2007) shown that neem extract provides control fungal diseases through a metabolic change in plants including induction of phenol biosynthesis enzymes, antioxidant defensive enzymes and phenol accumulation. Also, *Balanites aegyptiaca* (L.) Del., which belongs to the Balanitaceae family has potential medicinal value (Abuthaki *et al.,* 2019). Its mesocarp is rich in saponins and found to have fungicidal, microbial and larvacidal effects (Zarroug *et al.,* 1990; Yadav and Panghal, 2010). This research aimed to assess the antifungal potential of biochemical compounds of *Azadirachta indica* and *Balanites aegyptiaca* seed extracts identified by GC-MS analytical method against *F. oxysporum.*

MATERIALS AND METHODS

***Isolation of pathogen*:** *Fusarium oxysporum* f.sp. *lycopersici* was isolated from naturally infected root, stem and leaves of tomato collected in experimental fields at Monatele locality (4°12 N, 11°24 E). Infected plants were placed inside plastic bags and immediately taken to the laboratory. Different parts of the symptomatic plants were cut and washed with sterile distilled water, followed by blotting of excess moisture with a sterile blotting paper. Small pieces (3 mm) of organ were disinfected with sodium hypochlorite (10%) solution for 2 min. Potato dextrose agar (PDA) amended with ampicillin (250 mg/L) and nystatin (20 mg/L) was used to isolate fungus by depositing it on infected small pieces of root, stem and leaves. After 7 days incubation at 28°C, the mycelium that emerged was subcultured on PDA medium to obtain a pure explant (Hibar *et al.,* 2007). Furthermore, morphological characteristics were identified as well and microscopic examination were carried out for mycelia and conidia based on the method used by Chen and Swart (2001), and key determination presented by Booth (1977); Nelson *et al.* (1983).

***Preparation of Azadirahta indica and Balanites aegyptiaca extracts*:** Fruits of *Azadirahta indica* and *Balanites aegyptiaca* were obtained from Mora locality (4° 12 N, 11° 24 E) in the North region of Cameroon after scientific authentication by the Botanical Survey of Department of Plant Biology at the University of Yaounde I. Seed kernel were grounded using hand mill. 250 g of *A. indica* and *B. aegyptiaca* of powder were macerated in one liter of methanol and acetone solvent for up to 48 h. After maceration, the solution was filtered using filter paper (Whatman No. 1), and the filtrate was eliminated in a rotary evaporator (Büchi R-200 Rotary Evaporator at 60 °C). The extract obtained after evaporation was stored in the refrigerator at 4 °C for until use (Vongsak *et al.,* 2013). The aqueous solution was obtained by macerating 50 g of powder of *A. indica* and *B. aegyptiaca* into 100 mL of sterile distilled water for up to 48 h. The solution was filtered and used directly (Zibbu and Batra, 2010).

***GC-MS analysis*:** Acetone, methanol and aqueous extracts were analyzed using GC-MS method, that is gas chromatograph (Agilent GC 7890A) coupled with mass spectrometer detector (Agilent 5975 C TAD VL MSD) equipped with Column Elite-1 fused silica capillary column (30 m x 0.25 mm with 0.25 µm film thickness, composed of 5% phenyl methyl siloxane) and helium as a carrier gas. An electron ionization system with an ionization energy of 70 eV was used to detect compounds. The solution injected was prepared by mixing 1.5 g of anhydrous sodium acetate and 6 g of magnesium sulfate previously autoclaved and cooled in a desiccator with 10 mL for aqueous extract. For organic extract one milliliter (1 mL) was mixed with 2 mL of solvent (acetone or methanol) and 0.15 g magnesium sulfate. The mixtures obtained were vortexed and centrifuged at 4000 rpm. One microliter (1 µL) was injected into the column. The oven temperature was programmed at an initial temperature of 150 °C to hold for 1 min at 20 °C/min to a final temperature of 280 °C to hold for 9 min. Total GC running time was 55.44 min. The constituents were identified by comparing their spectra using the National Institute Standards and Technology (NIST) database with more than 62,000 patterns.

***In vitro antifungal activity assay*:** A stock solution of 500 µL/mL (mixing 50 mL of each extract with 100 mL of solvent

or water) was prepared. Then, 0.75; 1.5 and 3 mL of each stock solution were added to 29.25; 28.5 and 27 mL of PDA medium, respectively, for a final volume of 30 mL, which is poured into 90 mm Petri dishes at a rate of 10 mL per dish. Synthetic fungicide (Banko plus, active ingredient Chlorothalonil 550 g/L and Carbendazine 100 g/L) was used at the recommended concentration of 3.33 µL/mL and control. Mycelial hyphal from 7 to 10 days old pure cultures of *F. oxysporum* were plugged using 7 mm diameter and placed in the center of each Petri dish solidified by PDA medium at 3 different concentrations (15, 30 and 60 µL/mL) of aqueous extract (AqE), methanol (ME) and acetone extract (AE). There were three replicates of each treatment. Petri dishes were incubated in a growing chamber at 20 ºC, mycelial growth was measured every 2 days starting on the second day after incubation and ending when the hyphal development in the control was complete. The inhibition percentage of mycelial growth related to the control was calculated for all concentrations of each fungicide as formula proposed by Dissanayake (2014).

$$I\ (\%) = (Mc - Mt/Mc) \times 100$$

Where: I = inhibition percentage; Mc = mycelial growth of control; Mt = mycelial growth in the treatment.

***Determination of Minimum Inhibitory Concentrations*:** The minimum inhibiting concentrations of mycelial growth (MIC_{50} and MIC_{90}) of the different treatments used against *F. oxysporum* isolate were determined by comparing the values of the inhibition percentage with those of the Neperian logarithm of the corresponding concentrations (Espana *et al.*, 2017).

$$I = f\ (\ln Ci).$$

The linear regression line of the type Y = ax + b from the function I = f (ln Ci) thus made it possible to determine the MIC_{50} and MIC_{90} (Griffin *et al.* 2000).

Where: Y = inhibition rate (%), a = slope of the line, b = constant and $MIC = e^{x}$.

***Fungicidal or fungistatic test of the extracts*:** Mycelial explants at the end of the experiment were collected from the Petri dishes where fungal growth was totally inhibited. These explants were deposited aseptically on PDA medium without seed extract and fungicide. After 3 to 6 days, depending on whether or not the fungus resumed growth, the starting substance (extract) was qualified as fungistatic or fungicidal, respectively (Hmiri *et al.*, 2019).

***Determination of possible mode of action of the promising extract*:** The best extract that inhibited the mycelial growth of fungi will be used to evaluate pathogen wall lysis and protein synthesis.

***Lytic activity of extract*:** To measure lytic activity of extract, the method performed by Limsuwan and Voravuthikunchai (2013); Mbekou *et al.* (2021) with modifications were used. A fungal suspension was prepared using 0.5 McFarland scale in 0.9% NaCl and 100 µL of promising extract was mixed into different tubes to have a Minimum Inhibitory Concentration of 0.5 MIC and 1 MIC in the medium. The mixture was incubated at 37°C while stirring and the absorbance at 620 nm was measured at four periods (0 h, 2 h, 4 h and 6 h). The relative absorbance (Ar) at different times was evaluated using the absorbance at zero hours in order to draw the Ar= f(t) curve. All assays were carried out in triplicate.

***Inhibition of protein synthesis*:** Indeed Upadhyay *et al.* (2008) protocol, a mixture of 9 mL potato dextrose broth (PDB) and 0.5 mL of fungal suspension (standardized to McFarland's 0.5 scale) was homogenized in five tubes. A minimal inhibiting concentrations of 1 MIC and 0.5 MIC were prepared by adding 0.1 mL of the promising extract to the mixture against a blank and incubated at 37°C (Flore *et al.*, 2023). The mixture was centrifuged at 5000 g for 2 minutes to recover the fungal cells, which were weighed and mixed with the lysis buffer at a rate of 40 mg of fungi per 500 mL buffer. After one hour's of incubation, the protein containing supernatant reader (FLUOstar Omega Microplate Reader) was used to perform the assay using Bradford's reagent. The lysis buffer constituted the blank.

Statistical analysis: Data collected were subjected to one-way analysis of variance (ANOVA) using the R software version 4.0.4 (R development Core Team 2022). Significant differences between means were compared by Tukey's multiple range test (HSD) at 0.05. The regression linear and minimum inhibiting concentrations at 50% and 90% of mycelial growth (MIC_{50} and MIC_{90}) were determined.

RESULTS

***Isolation and morphological identification of Fusarium oxysporum*:** The fungal isolate was identified morphologically with the colonies grown on the PDA medium. Macroscopic (Fig 1A) characters of pure isolates in Petri dishes show colonies of white mycelial with a cottony appearance. Microscopic observation of the mycelial filaments under the light microscope shows isolated microconidia with zero to one oval to cylindrical septum and macroconidia with three to four medium to sickle-shaped or elongated septa (Fig. 1B).

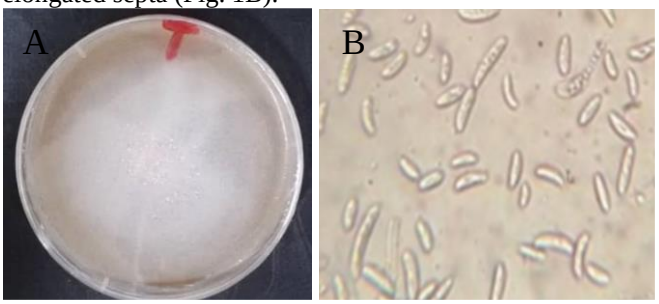


Figure 1. *Fusarium oxysporum* isolated from a naturally infected tomato plants organ collected at Monatele locality (4°12 N, 11°24 E); A: Pure isolate in PDA medium; B: Conidia.

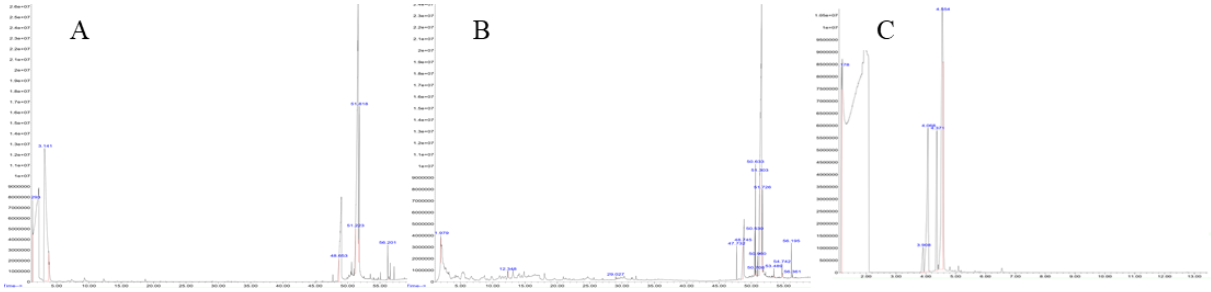


Figure 2. GC-MS chromatogram of Acetone (A), methanol (B) and aqueous (C) extracts of *Azadirachta indica* seeds.

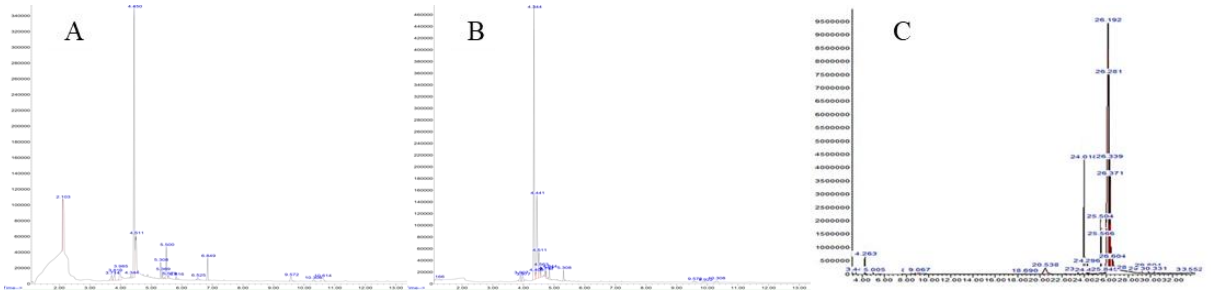


Figure 3. GC-MS chromatogram of Acetone (A) methanol (B) and aqueous (C) extracts of *Balanites aegyptiaca* seeds.

***Gas chromatography-mass spectrometry (GC-MS) of extracts*:** The acetone, methanol and aqueous extracts of *Azadirachta indica* and *Balanites aegyptiaca* seeds show the presence of numerous chemical compounds represented by the majority, minority, and ultra-minority peaks (Fig. 2 & 3). A total of 55 compounds were identified in the *A. indica* seed extract with 11 compounds in the acetone extract, 33 in the methanol extract (Fig. 2B) and 11 in the aqueous extract (Fig. 2C). For the *B. aegyptiaca* seed extracts, a total of 34 compounds were identified with 12 compounds each in the acetone and methanol extracts (Fig. 3A & B) and 10 compounds in the aqueous extract (Fig. 3C). The retention time, structures and molecular weights of only the bioactive compounds with fungicidal activity of both plants were retained (Table 1 & 2).

***In vitro* antifungal activity**

Antifungal activity of Azadirachta indica extract against Fusarium oxysporum: There was a significant difference in mycelial growth inhibition among the different concentrations of extracts, fungicide and control (Fig. 4). At 8 Days after inoculation, the control plates had been entirely colonized by *F. oxysporum* while the plates containing fungicide who was inhibited mycelia growth by 100 % (Fig. 5). By increasing the concentration of the extract, inhibition increased significantly (F.value = 778.2, $P < 0.001$; F.value = 386.3, $P < 0.001$; F.value = 425.6, $P < 0.001$) in AqE, AE and ME, respectively. In AqE, the concentration of 60 µL/mL has inhibited mycelia growth by 82.99 %, followed concentration of 30 µL/mL (79.70 %). In AE, concentration of 60 µL/mL registered a mycelia inhibition of 52.54 % and in ME, a concentration of 60 µL/mL has inhibited mycelia growth at 100 % like fungicide.

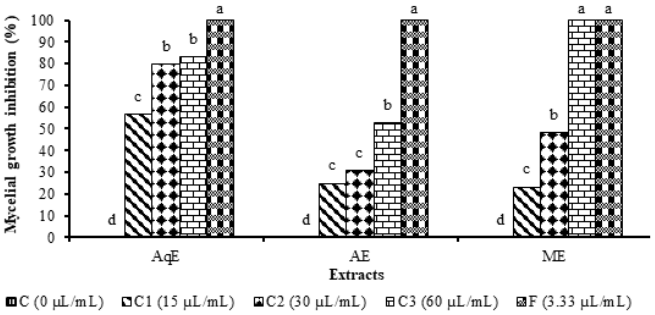


Figure 4. *In vitro* inhibition of mycelial growth of *Fusarium oxysporum* by *Azadirachta indica* extracts; AqE: aqueous extract; AE: *acetone* extract, ME: methanol extract; F: fungicide; C: control.

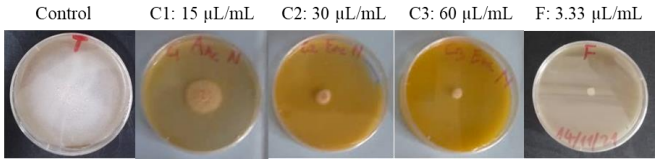
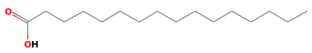
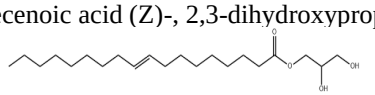
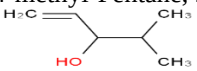
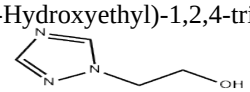
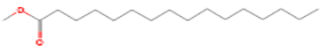
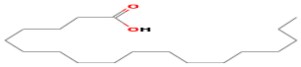
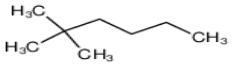
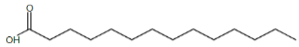
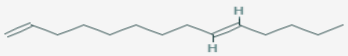


Figure 5. Inhibition of mycelial growth of *Fusarium oxysporum* by *Azadiratchta indica* with acetone extract.

***Antifungal activity of Balanites aegyptiaca extract against Fusarium oxysporum*:** At 8 Days after inoculation, it's

Table. 1. Summary of the GC-MS of *Azadirachta indica* seed extract compounds with antifungal activity.

Extract	Peak	Rt (min)	Area (%)	Mw	Name and Structure
AE	3	48.65	3.11	256.42	n-Hexadecanoic acid 
	4	51.22	4.98	280.45	9,12-Octadecadienoic acid (Z,Z) 
	6	56.20	2.09	356.54	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester 
ME	1	1.98	5.58	100.16	1-Penten-3-ol, 4-methyl-Pentane, 2,2,4-trimethyl- 
	2	12.35	1.54	114.23	1-(2-Hydroxyethyl)-1,2,4-triazole 
	4	47.73	4.72	155.19	Hexadecanoic acid, methyl ester 
	10	51.30	35.38	296.49	cis-13-Octadecenoic acid 
	11	51.73	11.15	296.50	Octadecanoic acid 
AqE	1	1.18	15.10	114.23	Hexane, 2,2-dimethyl- 
	3	4.07	23.02	228.37	Tetradecanoic acid 
	5	4.54	45.58	194.36	E-1,9-Tetradecadiene 

Rt: retention time (min); Mw: molecular weight g/mol; AE: Acetone extract; ME: Methanol extract; AqE: Aqueous extract

observed that there were a significant difference (F.value = 436.4, $P < 0.001$; F.value = 628.7, $P < 0.001$; F.value = 217.2, $P < 0.001$) in AqE, AE and ME respectively in mycelial growth inhibition among the different concentrations of extracts, fungicide and control (Fig. 6). The control plates had been entirely colonized by *F. oxysporum* and the plates containing fungicide has inhibited mycelia growth by 100 % (Fig. 7). Acetone extract of *B. aegyptiaca* at different concentrations weakly inhibited the mycelia growth of *F. oxysporum* with 40.10, 21.57 and 9.39 %, respectively to 60, 30 and 15 µL/mL. However, in methanol extract the concentration of 60 µL/mL inhibited as well as a fungicide (100%), followed by a concentration of 30 µL/mL (97.21 %). In the AqE extract, an inhibition of 60.15, 52.54 and 43.65 % were obtained by concentrations 60, 30 and 15 µL/mL respectively.

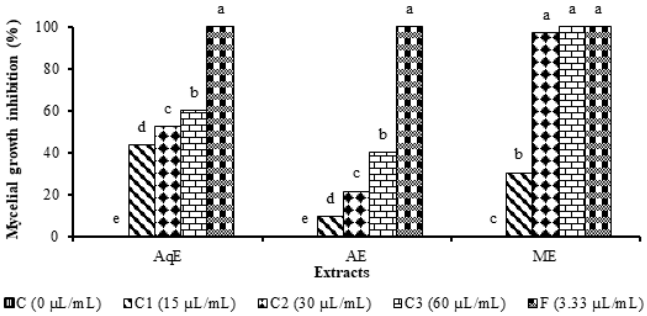
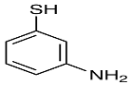
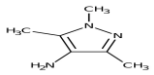
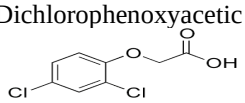
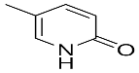
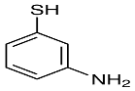
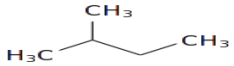
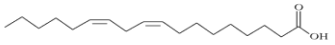


Figure 6. *In vitro* inhibition of mycelial growth of *Fusarium oxysporum* by *Balanites aegyptiaca* extracts; AqE: *aqueous* extract; AE: acetone extract, ME: methanol extract; F: fungicide; C: control.

Table 2. Summary of the GC-MS of *Balanites aegyptiaca* seed extract compounds with antifungal activity.

Extract	Peak	Rt (min)	Area (%)	Mw	Name and Structure
AE	1	2.10	6.58	79.10	Pyridine 
	4	3.98	4.15	125.19	3-Aminobenzenethiol 
	5	5.25	3.56	125.17	1,3,5-Trimethyl-1H-pyrazol-4-amine 
ME	1	1.16	1.59	221.04	2,4-Dichlorophenoxyacetic acid 
	2	3.97	1.97	109.13	2(1H)-Pyridinethione, 5-methyl- 
	4	4.34	52.05	79.10	Pyridine 
	5	4.41	1.11	125.19	3-Aminobenzenethiol 
AqE	1	4.27	1.67	72.15	Butane, 2-methyl- 
	6	26.15	38.27		9,12-Octadecadienoic acid (Z,Z)- 
	7	26.19	15.09	184.32	8-Dodecen-1-ol, (Z)- 

Rt: retention time (min); Mw: molecular weight g/mol; AE: Aceton extract; ME: Methanol extract; Aqueous extract

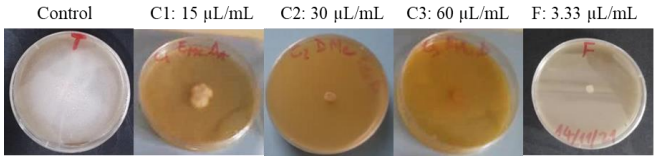


Figure 7. Inhibition of mycelial growth of *Fusarium oxysporum by Balanites aegyptiaca* with Methanol extracts.

***Minimal Inhibition Concentrations of the different extracts*:** The lowest minimal inhibitory concentrations (MIC50) were obtained with an aqueous extract (8.77 µL/mL) of *A. indica*, followed by the methanol extract (17.91 µL/mL) of *B. aegyptiaca*. For MIC 90, low minimal inhibitory concentrations of 39.75 and 54.28 µL/mL were obtained for the methanol extract of *B. aegyptiaca* and *A. indica* respectively. The highest MIC50 and MIC90 were registered with the acetone extract (98.39 µL/mL) and aqueous extract (598.56 µL/mL) respectively, for *B. aegyptiaca* (Table 3).

Table 3. Minimal inhibition concentration CMI 50 et CMI 90.

Plants	Extracts	CMI50	CMI90
Azadirachta indica	Methanol extract	26.39	54.28
	Acetone extract	60.38	448.15
	Aqueous extract	8.77	73.18
Balanites aegyptiaca	Methanol extract	17.91	39.75
	Acetone extract	98.39	598.56
	Aqueous extract	25.12	724.12

***Fungicidal or fungistatic activity of the extracts*:** The fungicidal and fungistatic effect of *Azadirachta indica* and

Balanites aegyptiaca extracts was presented in Table 4. Indeed, no growth resumption of *F. oxysporum* was observed after 6 days of incubation of methanol (ME) and aqueous (AqE) extract of *A. indica* and *B. aegyptiaca* which were found fungicidal. On the other hand, the acetone extract (AE) of *A. indica* and *B. aegyptiaca* was found to be a fungistatic effect.

Table 4. Fungicidal and fungistatic effect of extracts.

Plants	Extracts (60 µL/mL)	Effect
Azadirachta indica	ME	Fungicidal
	AE	Fungistatic
	AqE	Fungicidal
Balanites aegyptiaca	ME	Fungicidal
	AE	Fungistatic
	AqE	Fungicidal

Mode of action of promising extract

***Inhibition of pathogen wall synthesis*:** The lytic activity of *Azadiracta indica* and *Balanites aegyptiaca* methanol seed extract at the highest concentration at 60 µL/mL against *Fusarium oxysporum* varied with time (Fig. 8). The extract from *A. indica* and *B. aegyptiaca* seeds at 1 and 0.5 MICs lysed the pathogen's wall, in contrast to the control.

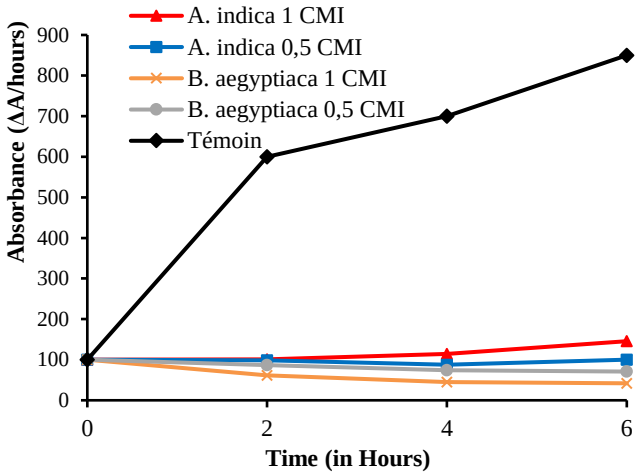


Figure 8. Lytic activity of *Azadiracta indica* and *Balanites aegyptiaca* seed extracts at 60 µL/mL concentration on *Fusarium oxysporum*.

***Inhibition of protein synthesis*:** The effect of *Azadiracta indica* and *Balanites aegyptiaca* extracts at the highest concentration of 60 µL/mL against *Fusarium oxysporum* shows that the extracts inhibited protein synthesis at 1 MIC and 0.5 MIC (Fig. 9). At 1 MIC, inhibition of protein synthesis induced by *A. indica* extract was 57.29% and for *B. aegyptiaca* 65.45%.

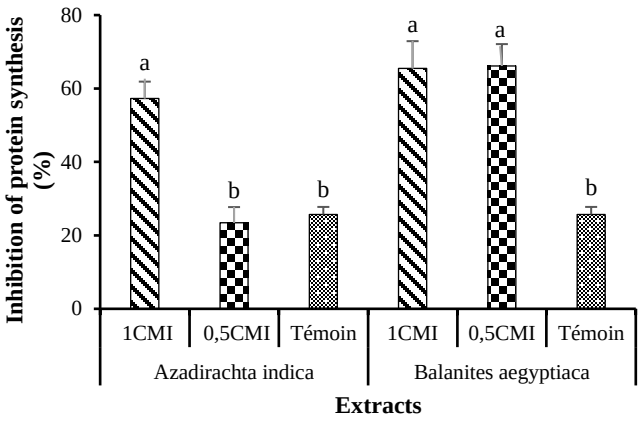


Figure 9. Effect of *Azadiracta indica* and *Balanites aegyptiaca* seed extracts on protein synthesis.

DISCUSSION

Morphological observation of *F. oxysporum* causal agent of tomato wilt was made based on macroscopic and microscopic characteristics. Macroscopic characters showed colonies of mycelium of white color with a cottony appearance. According to Nelson *et al.* (1994); Robles-Carrión *et al.* (2016); Arellano (2018), mycelial colors of *F. oxysporum* observed after growth on PDA medium were cottony, slow growth, with irregular shape, sparse, purple in the center and white at the edges. Microscopic observation shows microconidia and macroconidia. Indeed, Sun *et al.* (2018) have shown that the pathogen produces microconidia formed of one or two cells, macroconidia of three to five cells that gradually thin out, and chlamydospores consisting of one or two cells, which are thick-walled. *F. oxysporum* is a very destructive fungal pathogen that causes heavy losses in tomatoes.

Several methods including host resistance, cultural practice, and biological control are alternatives to chemical methods in the management of tomato wilt. Natural products obtained from plants are an effective option. GC-MS analysis of *Azadirachta indica* and *Balanites aegyptiaca* in acetone, methanol and aqueous extracts shows a chromatogram profile with majority, minority, and ultra-minority peaks. Results reveal three bioactive compounds with fungicidal activity in acetone, five in methanol and three in aqueous extract of *A. indica*. Concerning *B. aegyptiaca*, three compounds were found in acetone, four compounds in methanol extract and three in aqueous extract. These bioactive compounds present in *A. indica* and *B. aegyptiaca* seed extracts have been demonstrated by several studies to have antifungal activity, antimicrobial, insecticidal, and antibacterial. Adeshina *et al.* (2011); Rubila and Ranghanathan (2014); VasudhaUdupa *et al.* (2021) explain the importance of *Azadirachta indica* (Neem) with anti-inflammatory, hepatoprotective, antidiabetic activity, anti-nephrotoxic effect, neuroprotective

effects, antimicrobial, immunomodulatory, growth-promoting effect, antifungal, antioxidant and anticancer. Also, antimicrobial, antibacterial, larvicidal, antidiabetic and antifungal activity of seeds extract of *B. aegyptiaca* were demonstrated by Kame *et al.* (1991); Chapagain and Wiesman (2005); Awad *et al.* (2013); Habieballa *et al.* (2021).

The results of this study indicate that extracts of both tested plants show an inhibition on the mycelial growth of *F. oxysporum*. This inhibition may be due to bioactive compounds in these extracts (acetone, methanol and aqueous). The antifungal activities of *A. indica* and *B. aegyptiaca* have been tested against other pathogens including *Candida albicans*, *Fusarium oxysporum*, *Colletotrichum capsici*, *Aspergillus flavus* (Lopez-Bote *et al.,* 1998; Kavitha *et al.,* 2014). The methanol extracts of the two plants significantly reduced *F. oxysporum* growth *in vitro*, followed by aqueous extracts. These antifungal activity shown by methanol and aqueous extracts could be conferred to the solubility properties of certain solvents which are more efficient in extracting important bioactive compounds than others. The methanol is one of them (Bakari *et al.,* 2017). Indeed, Hlokwe *et al.* (2018) working on Evaluating crude extracts of *Monsonia burkeana* and *Moringa oleifera* against Fusarium wilt of tomato shows that methanol extract reduces inhibition of mycelial growth of *F. oxysporum*. On the other hand, VasudhaUdupa *et al.* (2021) presented a high antifungal activity of the cold acetone extracts of madhuca against *F. oxysporum*, which had an inhibitory effect by nearly two folds to that of the systemic fungicide bavistin. Also, Doughari *et al.* (2007) showed that the organic extracts (acetone and ethanol) had higher activity compared to the aqueous extracts. The concentration of 60 µL/mL of all extracts of both plants inhibited at 100% the mycelial growth of *F. oxysporium* as well as a synthetic fungicide (Chlorothalonil 550 g/L and Carbendazine 100 g/L). The inhibition of mycelial growth of *F. oxysporum* by the extracts of both plants may be due to the phytochemical compounds. In general, plants with pesticide potential rich in some secondary metabolites (Saponins, tannins, phenols, anthraquinones and Alkaloids) were demonstrated as having antifungal activity (Ramírez-Gómez *et al.,* 2019; Zulbayu *et al.,* 2021; Sukdee, 2023). Rosen and Stein Gold (2016); Yusoff *et al.* (2020) show that secondary metabolites interfere with the ABC transport system; inhibit the synthesis of ergosterol, ATP and aminoacyl tRNA synthetase leading to cellular dysfunction and impair sterol metabolism, which eventually causes leakage from plasmolysis and cell lysis. Thus, the inhibition of mycelial growth could be due to the abundance of several bioactive compounds in extracts of both plants. Some studies reveal that n-Hexadecanoic acid, 9,12-Octadecadienoic acid (Z,Z), 1-Penten-3-ol,4-methyl-Pentane,2,2,4-trimethyl-,

Hexadecanoic acid, methyl ester, cis-13-Octadecenoic acid and Octadecanoic acid possess antifungal properties (Ali *et al.,* 2016; Alawode *et al.,* 2021; Vuerich *et al.,* 2023). According to Rongai *et al.* (2017), the specific mechanism of action of the chemical compounds in the plant extracts is unclear, but it is likely that these chemical compounds form complexes with polysaccharides and proteins associated with an external layer of fungal cells that may result in a destabilization of the function of cell membranes, resulting in death of the pathogen.

The lowest minimal inhibitory concentrations (MIC_{50}) were obtained with aqueous extract (8.77 µL/mL) of *A. indica*, followed by the methanol extract (17.91 µL/mL) of *B. aegyptiaca*. For MIC_{90}, low minimal inhibitory concentrations of 39.75 and 54.28 µL/mL were obtained for the methanol extract of *B. aegyptiaca* and *A. indica* respectively. These lower MICs confirm the efficacy of *A. indica* and *B. aegyptiaca* seed extracts which are rich in secondary metabolites. The inhibitory capacity of plant extracts is due to the existent differences in components and concentrations of the active compounds contained in the species (Jasso de Rodríguez *et al.,* 2006). Extracts at concentrations of 15 and 30 µL/mL showed a fungistatic activity compared to the concentration of 60 µL/mL which is fungicidal. Indeed, Abbad *et al.* (2023) show an antifungal effect against *Phytophthora infestans*, *Alternaria solani*, *Botrytis cinerea* and *Oïdium oxysporum* fungi after exposure to aqueous extracts of *Peganum harmala*, *Caralluma europaea*, *Eucalyptus globulus*, *Ocimum basilicum* and *Nerium oleander*.

Our result reveals that methanol extract contributes to lytic membrane and inhibition of the protein of *F. oxysporum*. Yoshimi *et al.* (2022) demonstrated that extracts of plants rich in phytochemical compounds inhibit the synthesis of β-1,3-glucan, an essential cell wall component in many fungi. Phytochemical compounds in plant extracts disrupt the biosynthesis of pathogen cell wall components, inhibiting cell wall synthesis (Da *et al.,* 2019).

***Conclusion*:** In conclusion, *Azadirachta indica* and *Balanites aegyptiaca* seed extract contains several volatile chemical compounds identified by GC-MS as having antifungal activity. These chemical compounds present in methanol, acetone and aqueous extracts of both plants have inhibited the mycelial growth of *Fusarium oxysporum* at all the concentrations tested. The promising extract (methanol) of both plants induced wall lysis and protein synthesis in *F. oxysporum*. The antifungal effect of the studied plant extracts recommends it as a promising candidate for biological control of fungal pathogens, thus limiting the overdependence on chemical fungicides.

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Editing; P.M.T. Tayo, N.W.T. Kuate, H. Boli, T.S. Atindo and, T. Tize, Review and Editing.

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***Consent for publication*:** Not applicable

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